

61537

THESIS for the DEGREE of M.D.

A STUDY into CERTAIN ASPECTS of the OPSONIC INDEX in PULMONARY
TUBERCULOSIS : The INFLUENCE EXERTED THEREON of TUBERCULIN in VARY-
ING AMOUNTS : the QUESTION of the UTILITY of and NECESSITY for OP-
SONIC TECHNIQUE in the THERAPEUTIC ADMINISTRATION of TUBERCULIN.

J. E. GORDON, M.B., Ch.B.,
Royal National Hospital for Consumption,
Ventnor,
Isle of Wight.



A STUDY into CERTAIN ASPECTS of the OPSONIC INDEX in PULMONARY TUBERCULOSIS : the INFLUENCE exerted thereon of TUBERCULIN in varying AMOUNTS : the QUESTION of the UTILITY of and NECESSITY for OPSONIC TECHNIQUE in the THERAPEUTIC ADMINISTRATION of TUBERCULIN.

In September, 1906 I was appointed Research Scholar to the Royal Victoria Hospital for Consumption, Edinburgh. This post I held for a year.

Under the supervision and with the assistance of Dr. Philip, I undertook a series of observations into certain aspects of the opsonic content of the blood in pulmonary tuberculosis.

The main purpose of the Research was the determination of this factor, as a guide to future dosage with tuberculin, an indication being desired of dose, how much, and time to inject.

The scheme of observations comprised in all, the daily examination of eight sera of tuberculous individuals with a view to estimating their opsonic value. These were divided into four groups.

Group /

Group I. Two non-injection cases.

- " II. Two cases treated with tuberculin
in small doses, at short intervals.
- " III. Two cases treated with tuberculin
in larger doses, at longer intervals.
- " IV. Two cases treated with injections,
controlled by the opsonic index.

Wright in the article in the "Lancet", May 9th 1903, page 1302, shews by diagrams, which I reproduce, the importance, in successful treatment by tuberculin, of achieving a cumulative positive phase and in maintaining a higher base line of resistance.

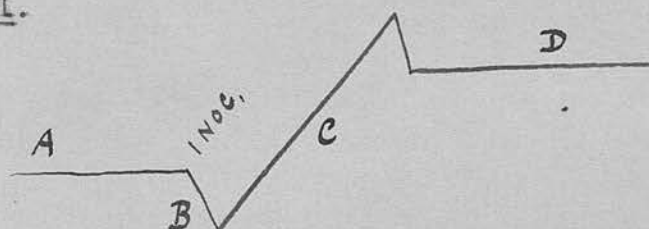
At this time, he was engaged in the agglutinin experiments upon the sera of tuberculous individuals undergoing inoculation with the new tuberculin (Koch T.R.) He laid importance on the agglutinins, as tests in the direction of forming an index to the obtaining of this cumulative positive phase.

More than 10 years previously Koch had given to the /

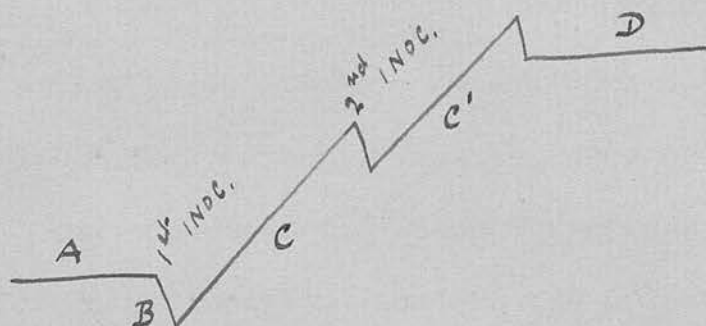
the world his tuberculin in the form of the old tuberculin (T.O.). The use of this agent, as a therapeutic, ended in disaster, pre-eminently, because, while the T.O. was unsuitable in treatment, the doses administered were excessive.

Wright from his experiments upon agglutinins, and using the new tuberculin for inoculation, endeavoured to formulate a rule for the "proper regulation and interspacing of successive doses."

Chart 1.



He shewed as in Chart 1., that if A was the resisting power before inoculation (ante tuberculin inoculation agglutinating power of blood on test fluid) an inoculation caused a negative phase at B (decline in resisting power) succeeded by a positive phase C. (increase in resisting power) and the final result was a higher base line of resistance at D.

Chart 2.

In Chart 2. the effect is shown of appropriate doses of vaccine and appropriate interspacing of doses. The second inoculation falls upon the positive phase caused by the first inoculation, and consequently a cumulation of increased resistive power is created.

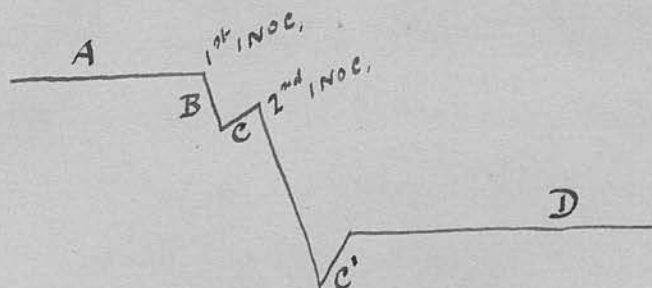
Chart 3.

Chart 3. exhibits the effect produced by two successive doses inappropriately interspaced. The second inoculation falls upon the negative phase of the first inoculation, and the result is a cumulation of negative phase or decrease in resistive power. A single inoculation of excessive dose has a similar effect.

The /

The agglutinins, however, as an index to vaccine inoculation in tuberculous infection, were not satisfactory. The advent of the opsonic theory elaborated by Wright and Douglas, and published in the Proceedings of the Royal Society, Vols. LXXII. and LXXIII, promised information on this point of a more reliable nature.

The importance of the appropriate quantum of tuberculin dose and interspacing of consecutive doses, was, in the case of tuberculosis, specifically demonstrated by these investigators before the British Medical Association in 1904. In the first place, by numerous ingenious experiments, they conclusively shewed the presence of bacteriotropic substances, to which they gave the name of opsonins, in the human blood and tissue fluids. In the second place, the notes of a series of cases undergoing therapeutic administration with tuberculin R. were exhibited.

The points they emphasised in these were :

(1) The continuous improvement recorded where the dose was not excessive, and where the proper interval between successive inoculations was maintained. That is to say, so long as the inoculation caught the positive /

positive phase of resistance, including the higher base line, a cumulation of increased resistive effect was created, and the conditions were favourable for a cure. The examples quoted, afforded confirmation of the theory.

(2) In the case of excessive dose or faulty interspacing between doses, the immunising power was lessened and consequently the clinical manifestations aggravated. An excessive dose caused a negative decline of the resistive factor of excessive duration and a succeeding inoculation of similar amount during this substratum contributed to further depression. Granted the quantum of dose was appropriate, adventitious interspacing of doses might result similarly, an inoculation during the primary negative fall of the preceding dose, producing cumulation of negative phase. Co-incident with this lessening in bacteriotropic value, the reparative processes tending towards healing of the disease, would desist, or spread and multiplication of lesions, and aggravation of clinical symptoms would ensue. In accordance with these theories, Wright exhibited several cases, where the tuberculo-opsonic /

opsonic content of the blood had not been determined, previous to or at the time of inoculation, with the result, that the injection had occurred during the negative fall of the preceding inoculation.

He laid particular stress, therefore, on the necessity of regulating administration of the vaccine by a determination of the opsonic index.

(3) Attention to amount and regulation of tuberculin inoculation may be ineffective with other infection, complicating the tuberculous disease, e.g. ^{staphylococcal} ~~staphylococcal~~. The vaccine appropriate to this organism must, therefore, be employed in addition.

(4) Standardising of Dose and Interval.

Originally, the doses of tuberculin R. employed by Wright varied from .002 to .1 milligram. Before the Royal Medical and Chirurgical Society, November, 28. 1905, Wright indicated, that the maximum of immunising effect can be obtained by small doses without constitutional disturbance, as is induced by large doses. $\frac{1}{1000} - \frac{1}{600}$ milligram are the limits, which Wright has most recently given in respect of dosage.

Further /

Further, while confirming the cumulation of negative phases with tubercle vaccine, he pointed out, at the same meeting, that cumulation in the direction of the positive phase must undergo modification. It is difficult, if not impossible, to achieve this. Wright is now content to treat each inoculation as an independent event.

Keeping these facts in view, the purpose of the Research outlined by Dr. Philip and carried out by me, was concerned mainly with the question of dose, and did not aim at confirmation of the experiments of Wright and Douglas. In the first place, the conditions governing the investigation were dissimilar in respect that, with two exceptions, the opsonic phenomena in pulmonary phthisis alone, were considered. Wright's researches covered the subject of tuberculosis as a whole, and more particularly, the subject of "surgical" tuberculosis or localised infection.

Secondly, the subjects of experiment were patients undergoing the ordinary hygienic-dietetic treatment of a consumption hospital. Concurrent with /

with clinical improvement due to these measures, there would be an increase of resistive power from natural causes, independent of artificial stimulation with tuberculin.

Thirdly, the doses employed, were minute, or minute to start with. So far as promoting the sequence of events, which are represented by the negative and positive phases, and resulting higher base line of resistance, they were usually ineffective. This phraseology, therefore, being unsuitable, it will be convenient to represent the variations in the opsonic content of the blood caused by the inoculation of the tubercle vaccine, by the terms subnormal, normal, and above normal. The significance of these terms will be better understood, when the charts come to be dealt with.

Fourthly, the medium used as an inoculating agent, was the tuberculin prepared by Professor Beraneck of Neuchatel. It may be well at the present time to indicate the composition and action of this preparation.

Beraneck's Tuberculin.

In /

In the true significance of the term, Beraneck's tuberculin cannot be called a vaccine. A vaccine is a sterilized and standardised suspension of micro-organisms. Nor is it an anti-toxin, in the sense, that Behring's anti-diphtheritic serum is. It is a mixture of two active toxins, the one derived from the bacilli themselves, and the other from the culture medium in which they are grown. The introduction of these into the system "cause an active infective process to counteract which, the organism manufactures an anti-toxin." The agent employed for the extraction of the toxin from the bacillary bioplasm is orthophosphoric acid.

Whatever may be the nature of the action of the anti-toxin elaborated in the tissues by inoculation with Beraneck's tuberculin, the tuberculo-opsonic content of the blood is favourably influenced by such inoculation.

Beraneck discovered his tuberculin in 1894. He has recently proved its success, especially in tubercular joint lesions.

Sahli of Berne, who has had several years experience of this agent, is convinced of its value "within /

"within certain limits and provided that proper precautions are taken in its administration."

Coulon at Neuchatel using graduated doses in cases of tuberculous bone lesions and general surgical tuberculosis, exhibited satisfactory results.

This therapeutic agent for over the past two years, has been used extensively, at the Royal Victoria Hospital, by Dr. Philip. In the department of pulmonary phthisis, it has been subjected to a searching enquiry, with results, which bear comparison with those obtained from the therapeutic administration of tuberculin R.

The scheme of dilutions devised by Dr. Philip comprises five grades, which are termed respectively, D1 or 1 in 10, D2 or 1 in 100, D3 or 1 in 1000, D4 or 1 in 10,000, D5 or 1 in 100,000. In making these dilutions, normal saline is employed without the addition of antiseptic or preservative. The tuberculin, as received from Beraneck, is regarded to be 100% strength or approximately pure, although it is much weaker than this. Solutions D1 and D2 are made directly from the "pure". The weaker dilutions are made /

made from a stock bottle of 1 in 100. The requisite quanta for dilution ^{are} is measured from a 1 c.c. pipette graduated into twentieths. The strictest aseptic precautions must be maintained in making the dilutions, and the same, of course, applies to the actual injection.

When it is desired to commence a series of inoculations, the procedure is, to give $\frac{1}{20}$ c.c. to $\frac{1}{10}$ c.c. D5, as the initial dose. The interval between successive doses is generally regulated by clinical manifestations in chest and temperature, pulse, aspect, etc. The dose is repeated in same strength or gradually increased, and with increasing dosage the intervals are lengthened. In course of time, the latter system leads to the administration of quanta of solutions of the strengths of D2 and D1 at weekly or fortnightly intervals.

While every satisfaction can usually be expressed with a clinical regulator in the therapeutic administration of tuberculin in pulmonary tuberculosis, as given upon the above method, the prospect of checking and confirming the readings of this regulator, and the /

the possibility of standardisation of dose and interval, by opsonic technique, necessarily influenced an investigation into opsonic principles.

VARYING CONDITIONS of EXPERIMENT.

The QUESTION of SUITABILITY of DOSE, How much and
INTERVAL between SUCCESSIVE DOSES.

Turning to the scheme elaborated for the elucidation of these points, the observations were grouped into four classes. The scope of these has already been briefly referred to.

Group I. TWO CASES of PULMONARY PHTHISIS UNDERGOING ORDINARY HYGIENIC - DIETETIC MEASURES WITHOUT INOCULATION TREATMENT. (See Charts 1 & 2 infra.)

As in the other cases, daily observations of the tuberculo-opsonic content of the blood were undertaken. These two cases were carefully chosen as types of mediumly advanced chronic pulmonary disease. They acted as a comparison with or control to the other groups receiving tuberculin injections.

Bulloch /.

Bulloch has given the normal index within the limits of .8 and 1.2, and Lawson and Stewart confirmed these figures. A reference to the charts dealing with these non-injection cases will reveal many stages in the opsonic curve, where the index is within these limits. Again, at other points, the resistive value is often subnormal or depressed, below .8. It is these subnormal readings and the tendency to oscillation of the index, which are representative of chronic pulmonary phthisis. The necessity, therefore, for frequent investigations of the index is apparent. A few odd examinations might catch the index occupying normal limits, and subnormal readings, be thus overlooked. The correct index can be learned from daily observations for a week or better a fortnight. The correct value of the index having been learned, subsequent observations can be less frequent.

In the latter event, the examinations at any one time should consist of daily estimations of the index over a period occupied by several consecutive days.

Bulloch /

Bulloch examined 14 cases of so called "sanatorium cures" or arrested cases of pulmonary tuberculosis, and the index was found depressed in all except one. These cases were all from the well-to-do classes and in at least one of these cases, which was considered a perfect cure, the disease has elapsed within a year.

Case 1.	O.I.	=	0.7
" 2.	"	=	0.6
" 3.	"	=	0.7
" 4.	"	=	0.6
" 5.	"	=	0.5
" 6.	"	=	0.4
" 7.	"	=	0.6
" 8.	"	=	0.65
" 9.	"	=	0.75
" 10.	"	=	0.6.
" 11.	"	=	0.5
" 12.	"	=	0.45
" 13.	"	=	0.6
" 14.	"	=	0.86

Contrasting /

Contrasting these figures with those obtained in the two cases under consideration, there is a distinct elevation in opsonic value in favour of the latter. One of these cases was a "cure", and while it agrees with Dr. Bulloch's investigations at many points, the index is often within normal limits and occasionally above normal. In the other, the disease in the chest was arrested, but it was not a "cure". The chart of this case compares favourably with the preceding. It is to be remembered, however, that these examinations were undertaken during treatment, and not after the patient had been discharged from hospital, for a shorter or longer period. It is conceivable, that co-incident with the fall in general wellbeing, which is often seen on the return of a phthisical patient from "sanatorium" life, there might be a corresponding fall in the resistive factor as measured by the opsonic index.

Group II. TWO CASES of PULMONARY TUBERCULOSIS, TREATED, in ADDITION to ORDINARY SANATORIUM TREATMENT by INOCULATIONS of TUBERCULIN in SMALL DOSES at SHORT and EQUAL INTERVALS. (See charts 3 & 4 infra.)

The effect of small quantities of tuberculin administered at 2 or 3 or 4 days intervals, upon the tuberculo-opsonic curve, is seen on reference to the charts accompanying the cases dealt with in this group. It was not proposed to control administration of the inoculations by the investigation of the index. Given a system of dosage and interspacing of successive doses, which had proved satisfactory from clinical observation, it was decided to test the behaviour of the opsonic index upon this system.

The cases selected to represent this group were as far as possible similar in respect of extent of pulmonary disease and constitutional disturbance to those cases illustrative of Group I. Under these circumstances, remembering the tendency to subnormal value and oscillation characteristic of the opsonic phenomena of Group I, conclusions with reference to the influence exerted on these factors by tuberculin administered in the manner exhibited, can be determined.

These conclusions are :-

(1) Increase in immunising power, as represented by elevation /

elevation of the opsonic index, one, two, or more points in the direction of normal value.

(2) Less tendency to fluctuation, the effect being an influence towards steadying of the oscillatory indices of Group I.

The combined effort results in an index, which approximates within normal limits.

Observers are generally agreed, that an index sustained indefinitely by tuberculin at a hypernormal value cannot be accomplished in pulmonary tubercle. Satisfaction then must be expressed with an index of hitherto subnormal tendencies, which can be caused by appropriate quanta of tuberculin inoculations to assume a normal reading. When one remembers the usual chronicity of pulmonary tuberculosis, and the long duration of treatment, it does not appear likely that stimulation by tuberculin of the immunising factor so as to obtain a high opsonic reading, for sufficient length of time to cure the disease, can be successful. On the other hand, could such a high phase be attained even for a short period, the disease might the more rapidly resolve. The high reading, as has been shewn from my own investigations and those of other workers, being unobtainable /

able in pulmonary tuberculosis, the successful attainment of the normal must be regarded as satisfactory.

Is the small dose, then favourable to this end ? Wright has stated, that the maximum effect of immunising power can be exhibited by small quantities of tuberculin. Large doses are unnecessary for this purpose, and may have the contrary effect, producing in short a negative phase. I think, on reference to the charts dealing with Group II, it will be conceded, that the small dose has been successful in the direction of fulfilling the expectations, which have been stated above as capable of realisation.

In regard to the question of length of interval between successive inoculations, satisfaction can be expressed with the results obtained by inoculations given at frequent and equal intervals (see charts 3 & 4 infra.). It may be argued, that if the interspace between these small inoculations were lengthened to say a week or 10 days, would there be any material lessening in value of the resistive curve ? In view of Wright's dictum that full immunisation can be created by a single small quantum of tuberculin, which /

which furthermore is of a prolonged effect, (lasting in the case of $\frac{1}{1000}$ mg. Koch's T.R. to from 10 days to 3 weeks) it seems reasonable to expect, that prolongation of these small intervals, the dose remaining the same, cannot result in any material decrease in the index value. In certain instances, where an inoculation was occasionally omitted, the opsonic record did not shew any alteration in the direction of subnormal.

The amount of dose being satisfactory, but interval being capable of prolongation, inoculations are now given on similar routine lines of small quantities of tuberculin at equal intervals, the latter being increased to 6 or 7 days.

Group III. TWO CASES of PULMONARY TUBERCULOSIS TREATED, in ADDITION to SANATORIUM TREATMENT, by INOCULATIONS of TUBERCULIN in MEDIUM or LARGE DOSES at EQUAL INTERVALS. (see charts 5 & 6 infra.)

One of the subjects dealt with in this investigation shewed extensive disease. The other case was moderately advanced. The object underlying this portion. /

portion of the research was to compare, with the foregoing groups, the influence exerted upon the opsonic index, by medium and large inoculations of tuberculin respectively.

The results achieved in this group are not so encouraging, while certain sections of the records contrast favourably with Group II, there is on the total reading a greater variation in regard to oscillation and subnormal value.. In the employment, therefore, of medium and strong dilutions of tuberculin, it is injudicious to commence dosage with these straightaway. The system of graduated doses must be used. Where the immunisation factor grows accustomed to, and fails to respond to the stimulus of the small quanta, it is necessary then to increase the dose; which increase is upon the lines already indicated in the section dealing with Beraneck's Tuberculin.

Group IV. TWO CASES of LOCALISED TUBERCULOUS DISEASE, TREATED, in ADDITION to SANATORIUM TREATMENT by INOCULATIONS of TUBERCULIN CONTROLLED by DAILY ESTIMATIONS of the OPSONIC INDEX. (See Charts 7 & 8 infra.)

The method employed of inoculating during a decline of the opsonic curve was successful in respect that, in the cases so treated, the disease was satisfactorily checked. These cases, however, were not cases of pulmonary disease, but of localised tuberculosis. There was slight apical disease in one of the subjects in addition.

What advantages are to be gained by this method over routine inoculation of small quantities of tuberculin at equal intervals, the index reading being disregarded? Taking into account, that the cases representative of this section, exhibited lesions of a localised nature, and that the subjects were otherwise robust, it must be said, that no outstanding advantage is gained. While the opsonic curves, compared with those of the preceding groups, shew apparently a better response to the inoculations, this is only what is to be expected in localised infection. It may be confidently stated, that charts equally satisfactory would result from injections administered at equal intervals. The quanta of inoculating fluid /

fluid given at any one time was never of sufficient potency to cause profound changes in the index, either in the negative or positive direction. Immunisation is nevertheless complete. The danger of marked depression from inoculation of a single small dose being negligible, successive doses of similar amounts can be exhibited at regular intervals with perfect safety.

The PRACTICAL UTILITY of and NECESSITY for
OPSONIC TECHNIQUE in the ADMINISTRATION THERAPEUTI-
CALLY of TUBERCLE VACCINE.

Sir A. E. Wright's discovery of the opsonic technique was the means of placing the therapeutic administration of tuberculin upon a sound and safe basis. In particular, it explained, the reasons of the previous unsuccessful treatment with tuberculin. It caused the abandonment of the large dose in favour of the small. The astonishing results, which can now be obtained by inoculation treatment in certain forms of tuberculosis are due to the discovery of opsonic principles. Nevertheless, these results can be /

be expected with confidence without at any time being under the necessity of employing this interesting but laborious process. The opsonic index has proved of extreme value in formulating certain institutes for vaccine therapeutics, e.g. the employment of tuberculin R. in small doses $\frac{1}{10,000}$ - $\frac{1}{1000}$ m.g. at intervals of not less than 10 days. Injections on these lines can be undertaken without opsonic control. The same applies to the use of other forms of tuberculin. In the case of Beranek's tuberculin, the doses being minute, the interval can be narrowed correspondingly. I have shewn in this research, that tuberculin administered on these lines, exerts upon the opsonic record no negative phase of any moment. The fact that tuberculin was successfully employed, upon the small dose principle, for two years at the Royal Victoria Hospital without opsonic technique : that the opsonic records of cases similarly treated, were satisfactory (charts 3 & 4 infra): that inoculations are given under control of occasional determinations of the index (which may vary very considerably in the /

the interim) with gratifying results, indicate that opsonic elaboration is not necessary. Tuberculin in larger amounts, however, requires in its administration a regulator of more delicate workmanship than the clinical manifestations may offer. The opsonic technique is, therefore, a necessary preliminary and accompaniment in these instances. A reference to Chart 6 will shew a prolonged substratum of opsonic value, due to over-dose. Clinical manifestations of excessive dose were delayed until the phase was well advanced, and the index, therefore, afforded the first indication, that the dose was excessive. On decreasing the dose, recovery took place.

Method.

In general, the modification introduced by Professor A. E. Wright, of Leishman's method, for the estimation of the phagocytic power of blood, was used. This requires :

(1) The blood serum of one, or better, that of several normal individuals, and of the tuberculous patient or patients.

(2) White blood corpuscles, plasma free.

(3) A dead and sterilised emulsion of tubercle bacilli.

Solutions.

(1) Neutral sodium citrate . . . 1%

Sodium chloride 75%

In sterile distilled water.

(2) Normal saline.

(3) Saline 1 in 1000.

For the collection of blood for serum, the back of the finger above the nail may be utilised. An elastic tourniquet constricts the finger above the point selected for pricking. The first drop of blood is wiped away. One end of Wright's curved tube is placed in the blood, which runs up and fills the bulb by capillary action. On heating and sealing the other end of the tube, a vacuum results on cooling, to fill which, the blood still further runs up the tube. The other end may then be sealed. Care must be taken not to overheat or singe the blood, as the opsonins are destroyed by a temperature of 60° C. The capsule of blood is then set aside to clot or clotting may be promoted in the incubator. This clotted blood on centrifugalising yields two layers, a lower stratum of clot, and an upper layer of clear serum. /

serum. The latter contains the opsonins. Instead of the curved tube, a capillary tube with a central bulbous expansion may be used and fulfils all requirements. Less blood is required with this tube, and it is not so apt to break in the centrifuge. Also over-heating and consequent destroying of the opsonins are minimised.

To obtain white blood cells, the finger may also be used. These may be obtained from any source, subjects of lymphatic blood diseases excepted. To prevent coagulation of the blood, solution I, which is a combination of sodium citrate and sodium chloride, is employed. Blood is added to the citrate solution in the proportion of 1 to 5. The calcium salts of the blood are precipitated by the citrate solution. The tube employed for collection of the white cells is a straight tube. The finger is pricked and the blood immediately transferred to the citrate solution. Mixing must be thorough. The white cells are separated by centrifugilisation. This takes about 10 minutes in a machine revolving 4000 per minute. The cells settle to the bottom of the tube /

tube, the upper layer of the deposit being represented by a whitish film of white corpuscles. The supernatant fluid is pipetted off, normal saline added, the whole shaken up again, and again centrifuged. This process may be repeated several times, the more frequent the washing, the more thoroughly the leucocytes are freed from serum. The top layer of the deposit, containing the white cells, is removed by a pipette to a watch glass.

For the emulsion, Allan and Hanbury's "Moist" is very satisfactory. A minute portion is finely ground up in an agate mortar, and suspended in 1 in 1000 saline. This dilution is less apt to cause clumping of the bacilli. Clumps are removed by centrifugalisation. The appropriate fineness of the bacillary suspension can only be learned by experience.

If it is desired to determine the tuberculo-opsomic content of 4 patients' sera, and say 2 normal sera are to be used as controls, then six observations will be required. This implies the measuring and mixing together of the three constituents, detailed above. Six fine long-drawn capillary pipettes are /

are necessary. 1 centimeter is marked by a grease pencil from the ends of each, which is the unit volume of each pipette. A rubber teat is fixed to the other end. Equal volumes of each fluid in the order, cells, emulsion, serum, are measured, a bubble of air separating the segments. No. 1. pipette having been so filled, the segments are thoroughly mixed and incorporated, the one with the other, and returned to the pipette, the end of which is sealed. The pipette is placed in the incubator at 37°C . No. 2. pipette is similarly treated with the exception, that No. 2. serum is received; and so on. Each capsule remains in the incubator 15 minutes. At the end of this time a drop of the contents of each pipette, after a second mixing, is transferred to a slide, and a film made. The slides are now ready for fixing, staining, and counting. Fixing by heat is quite satisfactory, if the accompanying staining formula be employed : heated Carbol-Fuchsin 4 min. wash 2% sulphuric acid twice, wash, 4% warm acetic acid, wash, weak ammonia water. Counter stain Loeffler Blue 4 - 5 min. The bacilli in 50 consecutive polymorphonuclear cells are counted. /

counted.

The opsonic index is :

$$\frac{\text{opsonic content of unit volume of patients' serum.}}{\text{Normal person's serum, which equals unity.}}$$

The number of bacteria in 50 consecutive cells of each of the patients' slides divided by the number in 50 cells of the normal slide gives the opsonic index of each respectively. Where several normal sera are used, the average count represents the number in the normal slide. In this research, the sera of two normal individuals were employed. Another method is to "pool" the serum from several healthy persons, the count of which "pooled" serum is the normal slide and represents unity.

DETAIL of CASES.

Group I.

The two following charts are those of non-injection cases. Latterly one of these patients was receiving tuberculin on account of an ischio-rectal abscess.

Chart /

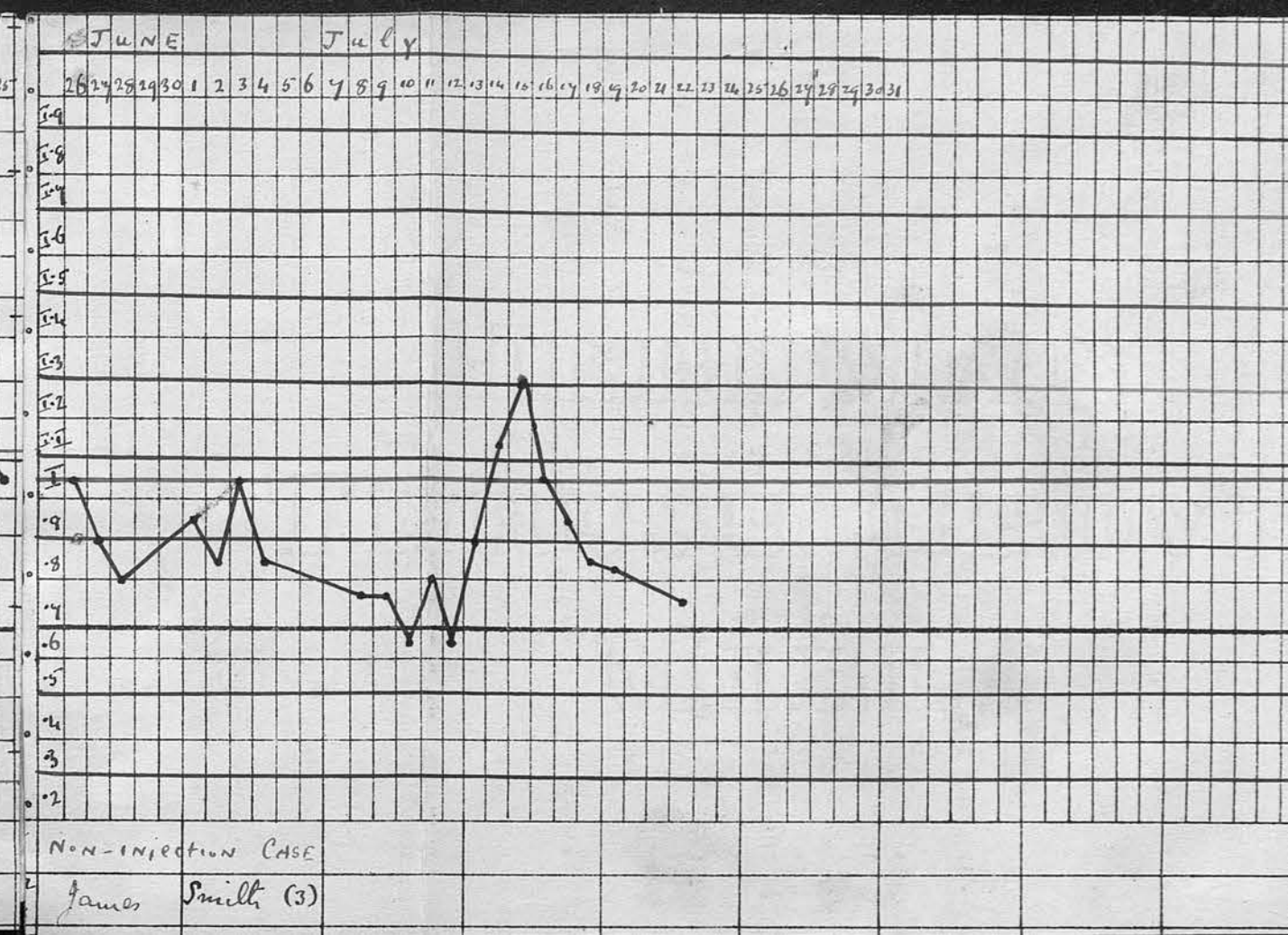
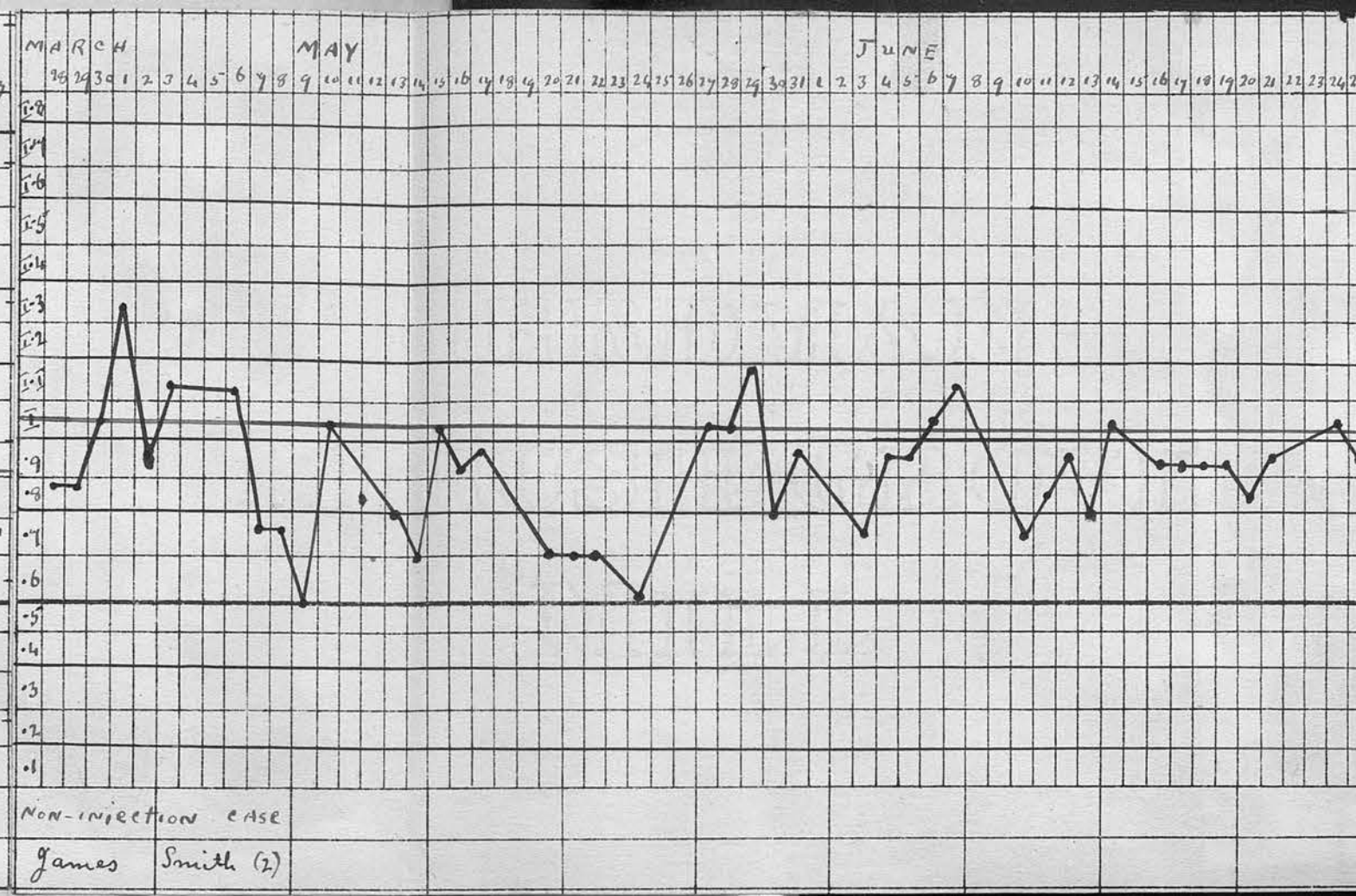
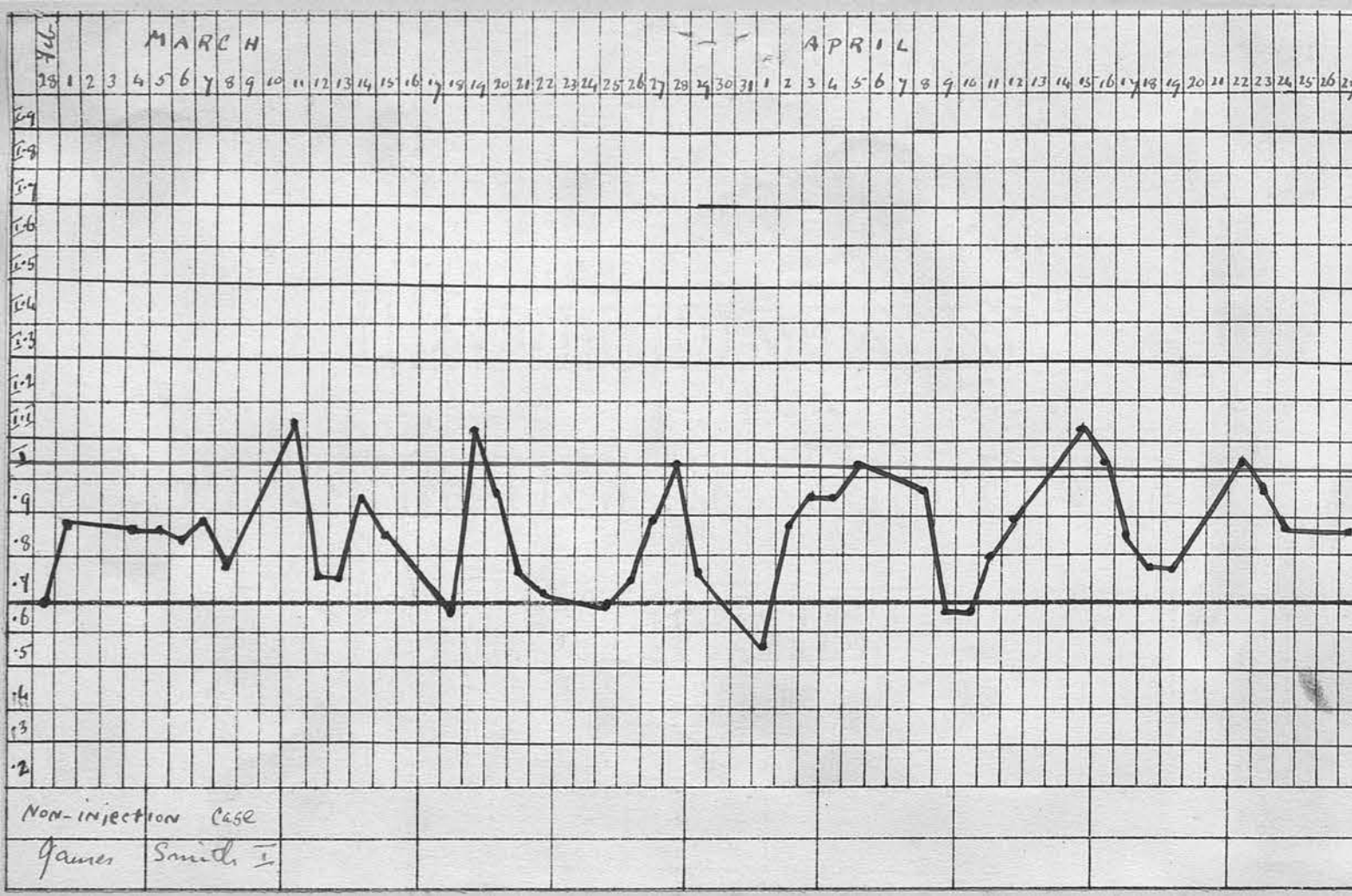
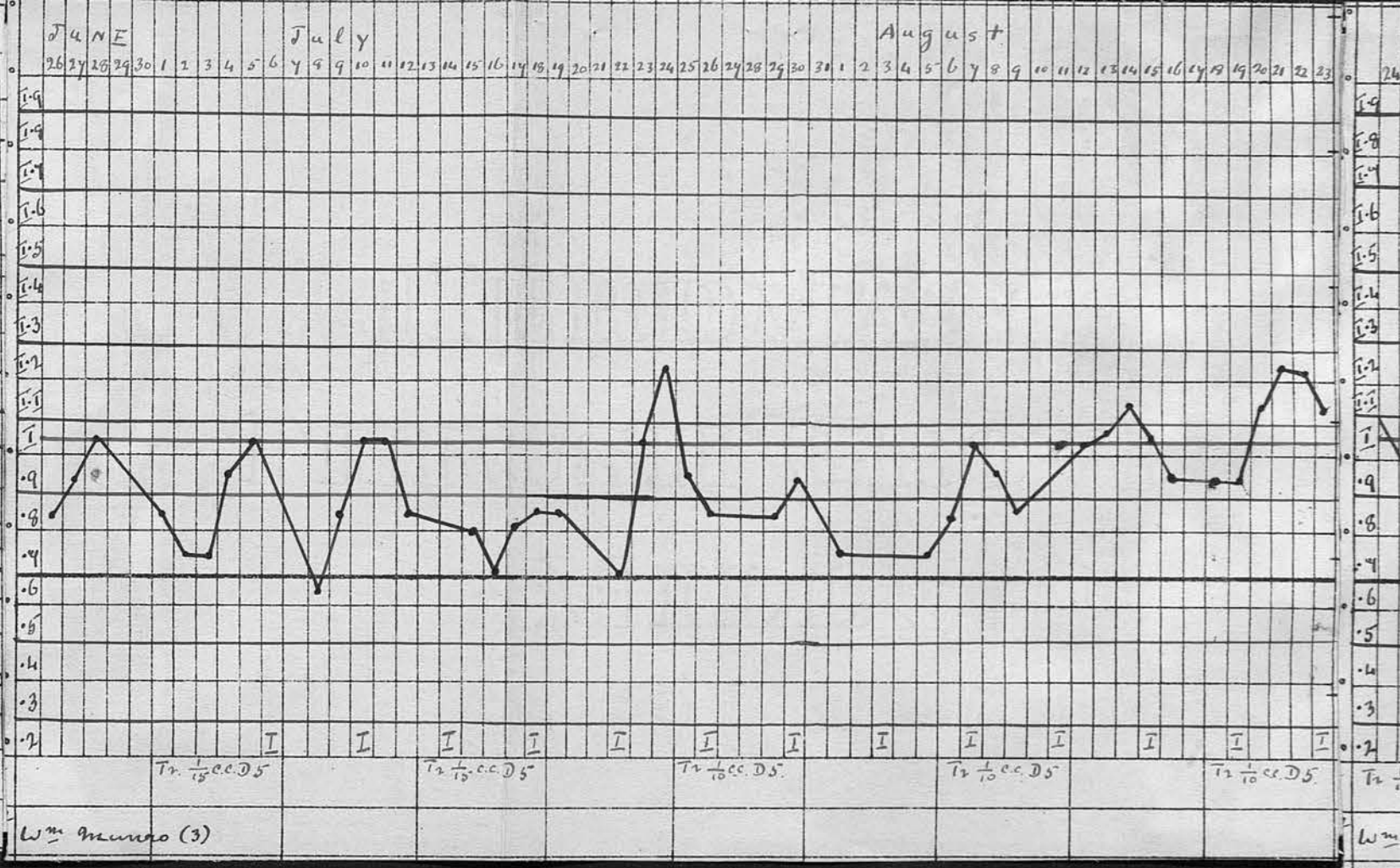
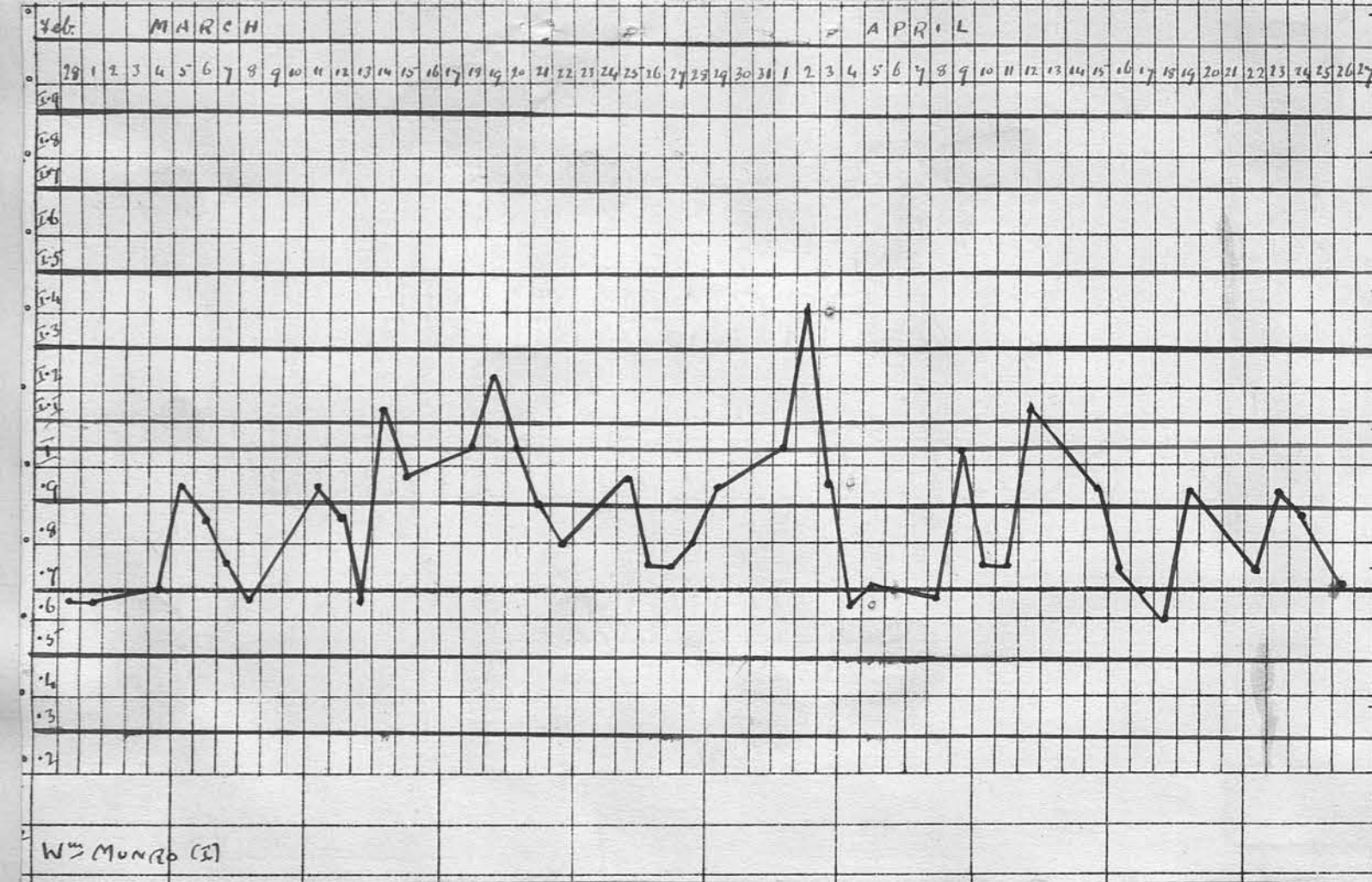


Chart I.

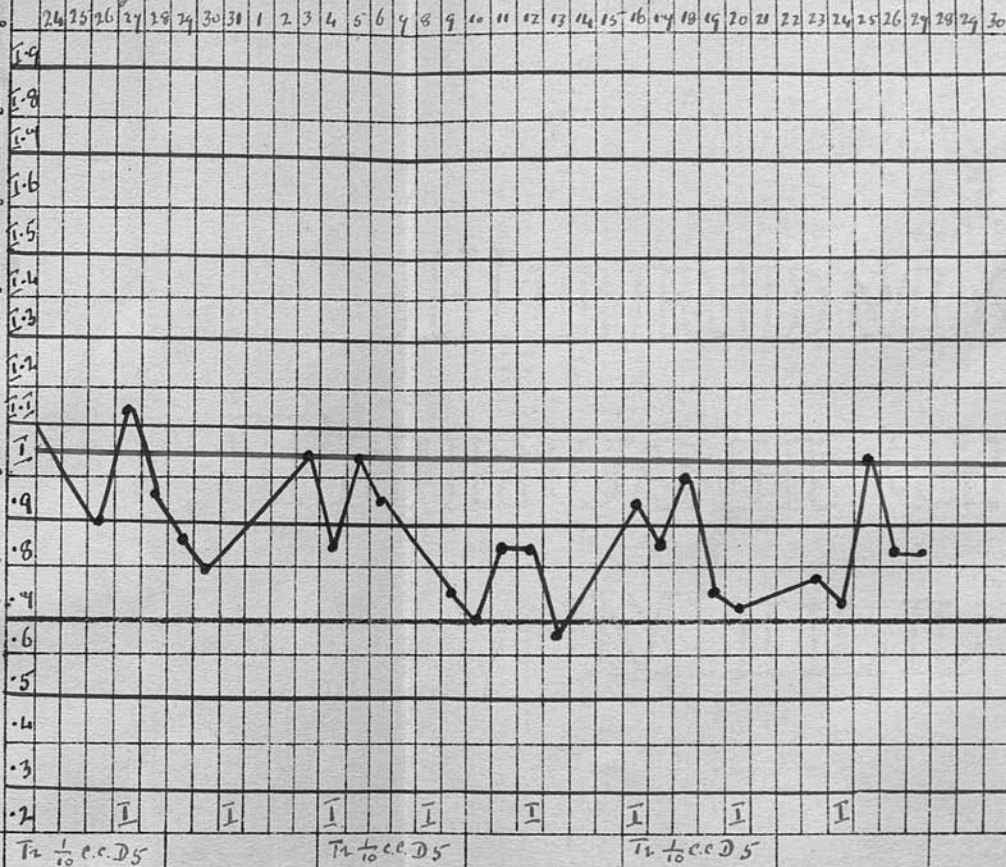
J. S. age 40, a car-driver was admitted into Hospital with extensive bilateral disease. Under the ordinary hygienic-dietetic measures, he made rapid improvement. He remained six months under supervision, being discharged with the disease completely checked and a gain of over two stones in weight. During the greater part of his stay, he was working from 4 to 6 hours daily. A study of the accompanying chart exhibits one or two points, which are of interest. In the first place, the variation which the index may shew in 24 hours is noteworthy. This patient latterly was apparently in the best of health. The signs of active disease were absent, the chest being dry, and the dullness greatly lessened in degree and extent. Temperature and pulse were always normal. Auto-inoculation or absorption of toxins from the pulmonary area could not be advanced to explain such departures as .55 one day and 1 or 1.1 the next. The futility, therefore, of forming an idea /

idea of the index from one observation must impress itself. If possible, daily observations for a week or a fortnight should be undertaken. The chart as a whole is fairly typical of chronic pulmonary tuberculosis. There is a tendency for the index to remain about .6, .7, and .8, with exacerbations into 1 and 1.1. It must be remarked, that while the clinical appearances and physical examination of this patient precluded any immediate extension of the trouble, the low tendency of his index under ordinary hygienic treatment suggested his liability to a recrudescence at any time. In other words, in spite of the marked improvement in him clinically, his opsonic value remained much as before. No opportunity was offered for finding the influence of tuberculin upon this reading. No further benefit could possibly have resulted in the clinical picture, with this addition to the treatment. In any case, the patient left Hospital unexpectedly, before injections were considered.



August

September



Wm Munro (4)

Chart 2.

W.M., age 26, suffered from bilateral tuberculo-
 sis of very considerable extent. Several years pre-
 viously, the knee joint was excised for tubercle.
 Residence in Hospital was about a year. Patient
 latterly was working about four hours daily. Com-
 pared with the former case, the opsonic reading pre-
 vious to the commencement of injections shews much
 the same characters. The disease in this patient
 was more extensive and of an inclination to advance-
 ment. Temperature was subnormal throughout. The
 signs of moisture and active disease were, however,
 never absent. Tuberculin was commenced early in
 July on account of the development of an ischio-rectal
 abscess. This discharged from 1 - 2 drams of pus per
diem. The small dose $\frac{1}{15}$ c.c. D5. was given and rapid-
 ly increased to $\frac{1}{10}$ c.c. D5., the intervals being three
 days. The primary effect of the injections was to
 steady the index to an appreciable degree. There was
 less oscillation. In the beginning of August a sus-
 tained rise in the record commenced, the maximum attain-
 ed being 1.2. At the end of the same month, the
 effort /

effort was exhausted. Thereafter the reading fell under control, but the swinging tendency, characteristic of the pre-injection period, was much less noticeable. My last observations on this case found the abscess smaller and the discharge of pus considerably less. Improvement in the condition coincided with the rise in the opsonic curve, which occurred in August. The physical sign in the chest remained unaltered by the tuberculin.

Group 2. The following represent the effect of injection of small doses at frequent intervals.

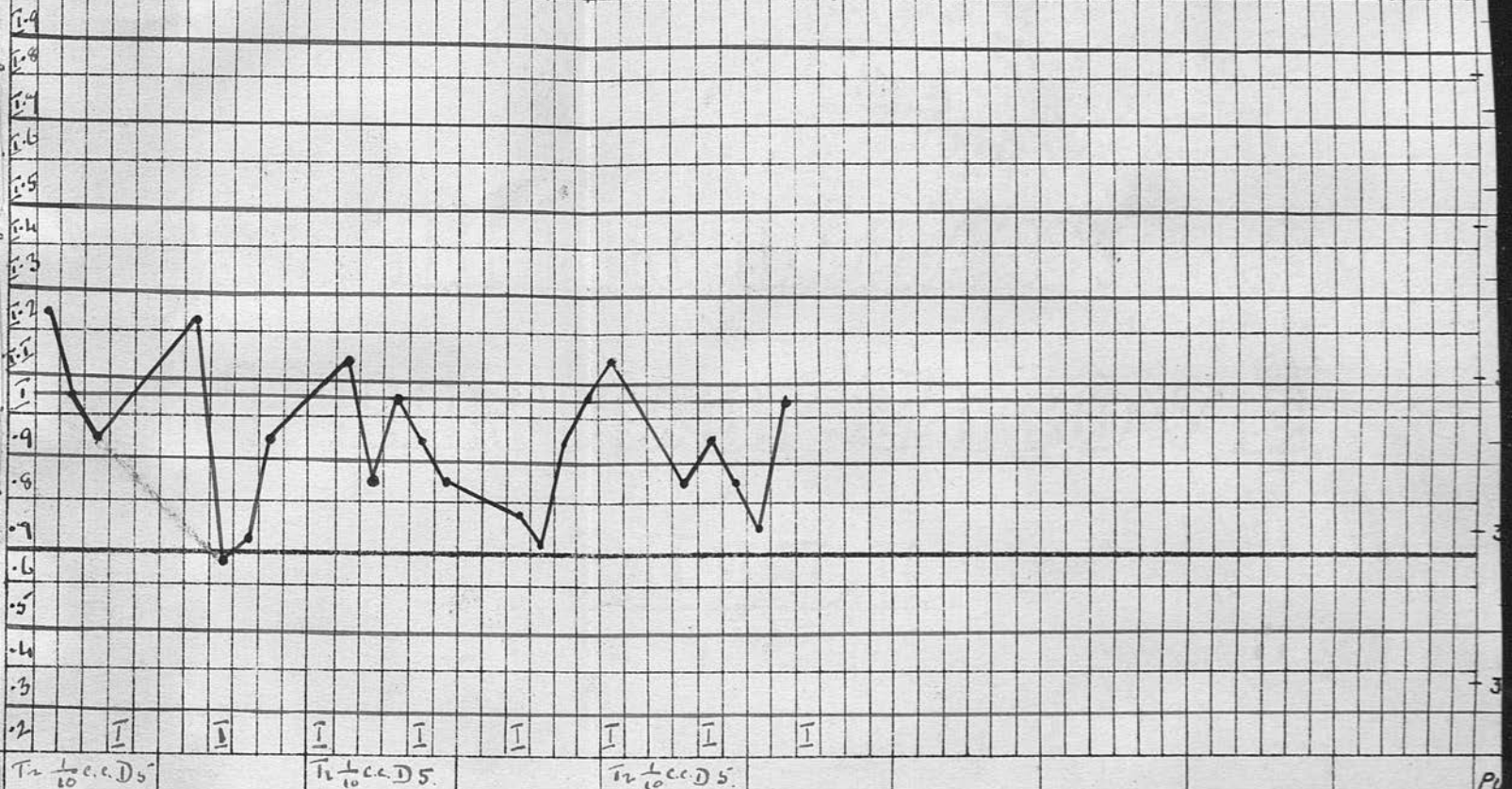
Chart 3.

M. F. age 17, was admitted into Hospital with extensive dullness and coarse moist creps on both sides, particularly left. Temperature on admission was very slightly elevated, but it quickly became subnormal. On day following second injection, there was a slight rise in temperature, from 96 to 99.2, On day following third injection to 99.6 and on day following fourth injection to 99.8. Thereafter temperature was unaffected by the vaccine. From the method /

AUGUST

SEPTEMBER

28 29 30 31 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30



A. MILNE	(4)
----------	-----

method of injection followed, the index throughout has been found to result satisfactorily. The oscillation often exhibited by the non-injection records is much less marked, a fairly constant reading from .7 to 1 resulting. The effect of these small doses, therefore, has been to steady the index and maintain it fairly near the normal or control. They have not caused at any time a high reading. In view of the fact, that the improvement, clinically, was satisfactory, this was never aimed at. Before injections were commenced, the physical signs in the chest were improving. They continued to do so during the course of injections. The general state mended rapidly. This was particularly noticeable in the constantly increasing gain in weight.

Chart 4.

A. M., age 45, came under observation with marked bilateral tuberculosis and accompanying constitutional disturbances. Temperature soon became subnormal. The dose employed was $\frac{1}{10}$ c.c. D5, double the strength of that given in the preceding instance.

The /

AUGUST

SEPTEMBER

24 25 26 27 28 29 30 31 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30

1.9

1.8

1.7

1.6

1.5

1.4

1.3

1.2

1.1

1.0

.9

.8

.7

.6

.5

.4

.3

.2

.1

0

-1

-2

-3

-4

-5

-6

-7

-8

-9

-10

-11

-12

-13

-14

-15

-16

-17

-18

-19

-20

-21

-22

-23

-24

-25

-26

-27

-28

-29

-30

-31

-32

-33

-34

-35

-36

-37

-38

-39

-40

-41

-42

-43

-44

-45

-46

-47

-48

-49

-50

-51

-52

-53

-54

-55

-56

-57

-58

-59

-60

-61

-62

-63

-64

-65

-66

-67

-68

-69

-70

-71

-72

-73

-74

-75

-76

-77

-78

-79

-80

-81

-82

-83

-84

-85

-86

-87

-88

-89

-90

-91

-92

-93

-94

-95

-96

-97

-98

-99

-100

-101

-102

-103

-104

-105

-106

-107

-108

-109

-110

-111

-112

-113

-114

-115

-116

-117

-118

-119

-120

-121

-122

-123

-124

-125

-126

-127

-128

-129

-130

-131

-132

-133

-134

-135

-136

-137

-138

-139

-140

-141

-142

-143

-144

-145

-146

-147

-148

-149

-150

-151

-152

-153

-154

-155

-156

-157

-158

-159

-160

-161

-162

-163

-164

-165

-166

-167

-168

-169

-170

-171

-172

-173

-174

-175

-176

-177

-178

-179

-180

-181

-182

-183

-184

-185

-186

-187

-188

-189

-190

-191

-192

-193

-194

-195

-196

-197

-198

-199

-200

-201

-202

-203

-204

-205

-206

-207

-208

-209

-210

-211

-212

-213

-214

-215

-216

-217

-218

-219

-220

-221

-222

-223

-224

-225

-226

-227

-228

-229

-230

-231

-232

-233

-234

-235

-236

-237

-238

-239

-240

-241

-242

-243

-244

-245

-246

-247

-248

-249

-250

-251

-252

-253

-254

-255

-256

-257

-258

-259

-260

-261

-262

-263

-264

-265

-266

-267

-268

-269

-270

-271

-272

-273

-274

-275

-276

-277

-278

-279

-280

-281

-282

-283

-284

-285

-286

-287

-288

-289

-290

-291

-292

-293

-294

-295

-296

-297

-298

-299

-300

-301

-302

-303

-304

-305

-306

-307

-308

-309

-310

-311

-312

-313

-314

-315

-316

-317

-318

-319

-320

-321

-322

-323

-324

-325

-326

-327

-328

-329

-330

-331

-332

-333

-334

-335

-336

-337

-338

-339

-340

-341

-342

-343

-344

The interval was longer, the injections being administered every third day. Compared with the former chart, the curve for the first two months is distinctly higher. There is, however, more oscillation. The succeeding stages represent a fairly constant value in the neighbourhood of control. Under the tuberculin, the physical signs cleared to a considerable degree. The improvement had been very encouraging under the Hospital regime. The addition of the vaccine to the treatment still further favoured the improvement.

Group III.

Injections of medium and large quantities are seen in the following. The intervals were a fortnight and week, respectively.

Chart 5.

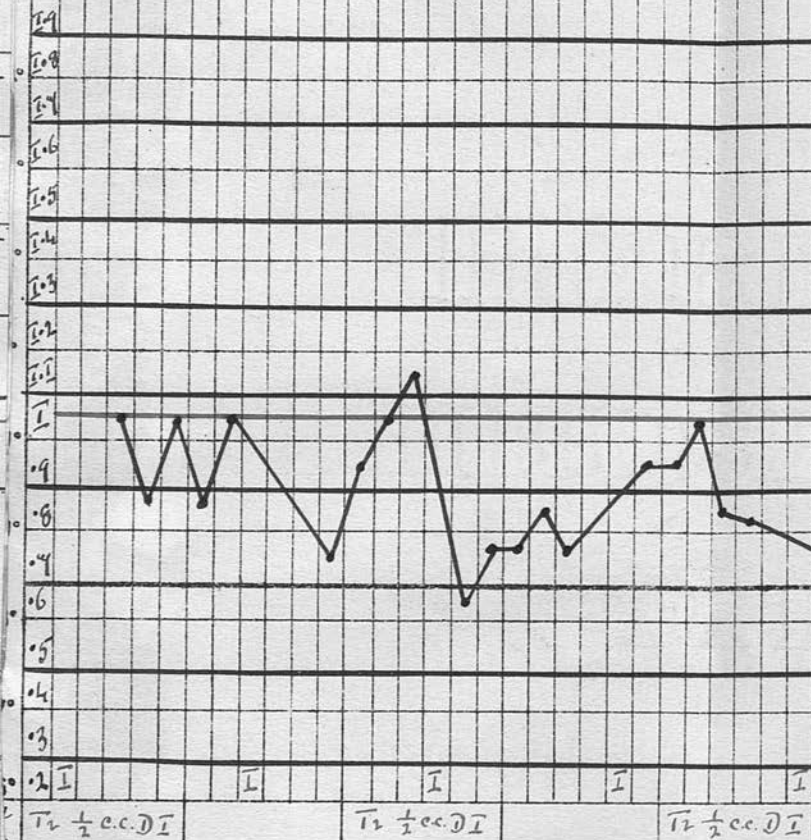
J.W. age 28, had severe bilateral disease, with moist crepitations, numerous over both lungs. Patient was a very frail subject. She was liable to occasional attacks of malaise from toxic absorption. Concurrent with these, the opsonic curve recorded marked /

marked oscillations, presumably from auto-inoculation. The influence of the injections upon these rises was probably minor. In any case, the index is a swinging one, with frequent excursions into .5. $\frac{1}{2}$ c.c. of D4 or solution 1 in 10,000 was injected fortnightly. The medium dose is not so successful in its results upon the opsonic record, as was the smaller. There is more oscillation and the total reading is less constant and decidedly lower. The fact, that this patient displayed more constitutional disturbance than the subjects of the smaller quantities must, of course, be taken into consideration. The pulmonary lesion was more severe. These in themselves would conduce, to a certain extent, to the shortcomings of this chart. On the 8th June, the dose was altered from $\frac{1}{2}$ c.c. D4 to 1 c.c. D5, the latter representing an injection five times weaker. Two injections of this quantity were given, and the intervals were the same as before. The result was an immediate steadying of the reading. The former dose $\frac{1}{2}$ c.c. D4 was returned to on August 1. with the previous vagaries of oscillation and depression to .6 and .5. The pulmonary condition remained stationary /

AUGUST

SEPTEMBER

24 25 26 27 28 29 30 31 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30



T. Johnston (4)

stationary under the tuberculin. Crepitations were never at any time absent. Patient was usually able to go about and undertake light duties.

Chart 6.

T. J. age 42, occupation a gardener, was admitted with severe bilateral disease. The chart compares favourably with chart 5, representing the medium dose. On the total record, it is inclined to be higher with less swing. Frequently, a low reading is recorded, as .5 and .6, and the oscillation at certain points, is pronounced.

The necessity for determining the index in such a case as this is obvious. While the lowest point in the chart is .5, the reading might at any time reach a lower level. The indication would then be to either decrease the dose or lengthen the intervals. Such an opportunity was afforded during the period commencing 13th May, to which, reference has already been made. The index shewing a tendency to prolonged depression, recovered on lessening the dose by one half. The chest latterly became clear, accompaniments disappearing. Dullness persisted as before.

Group IV.

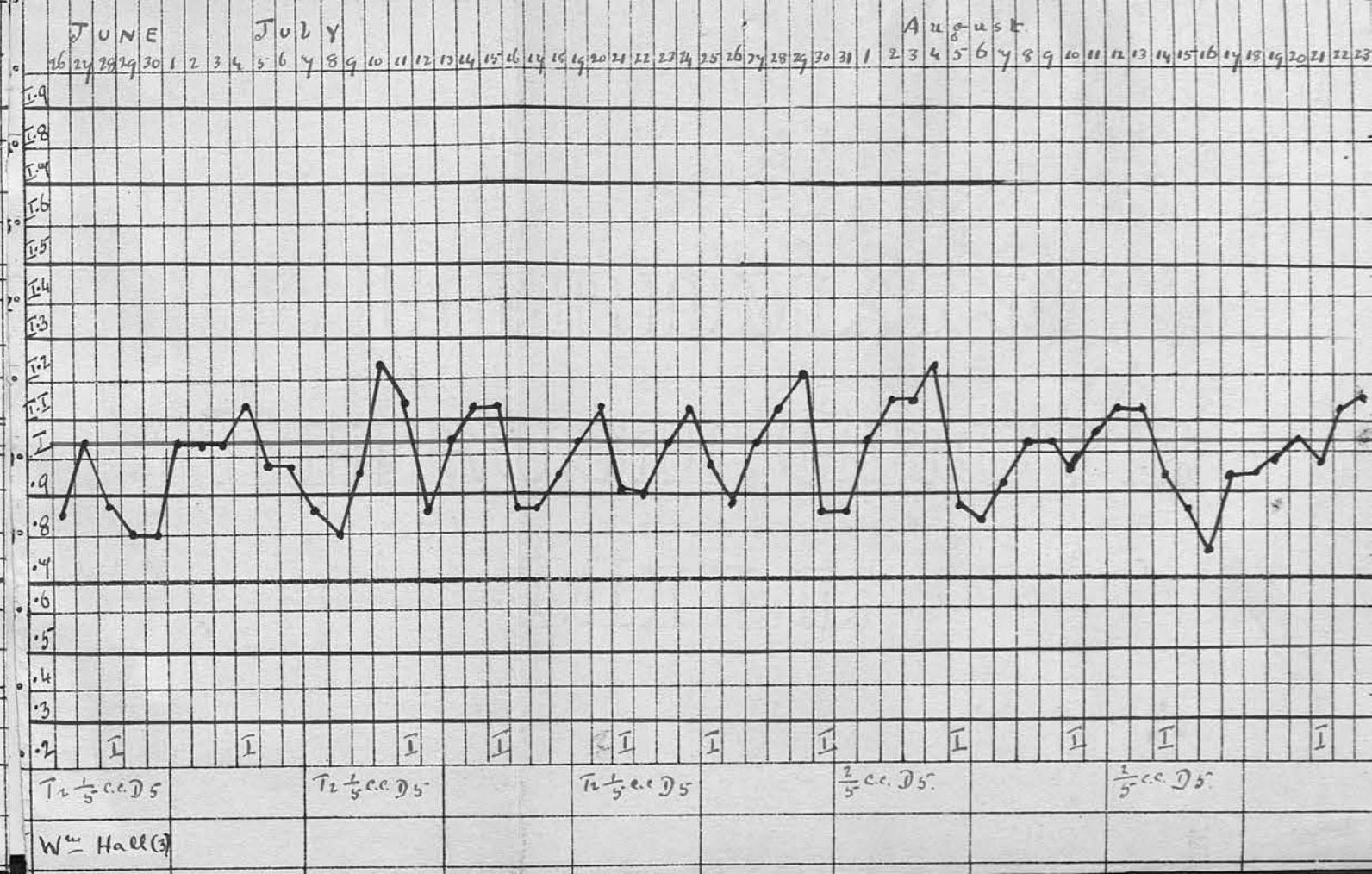
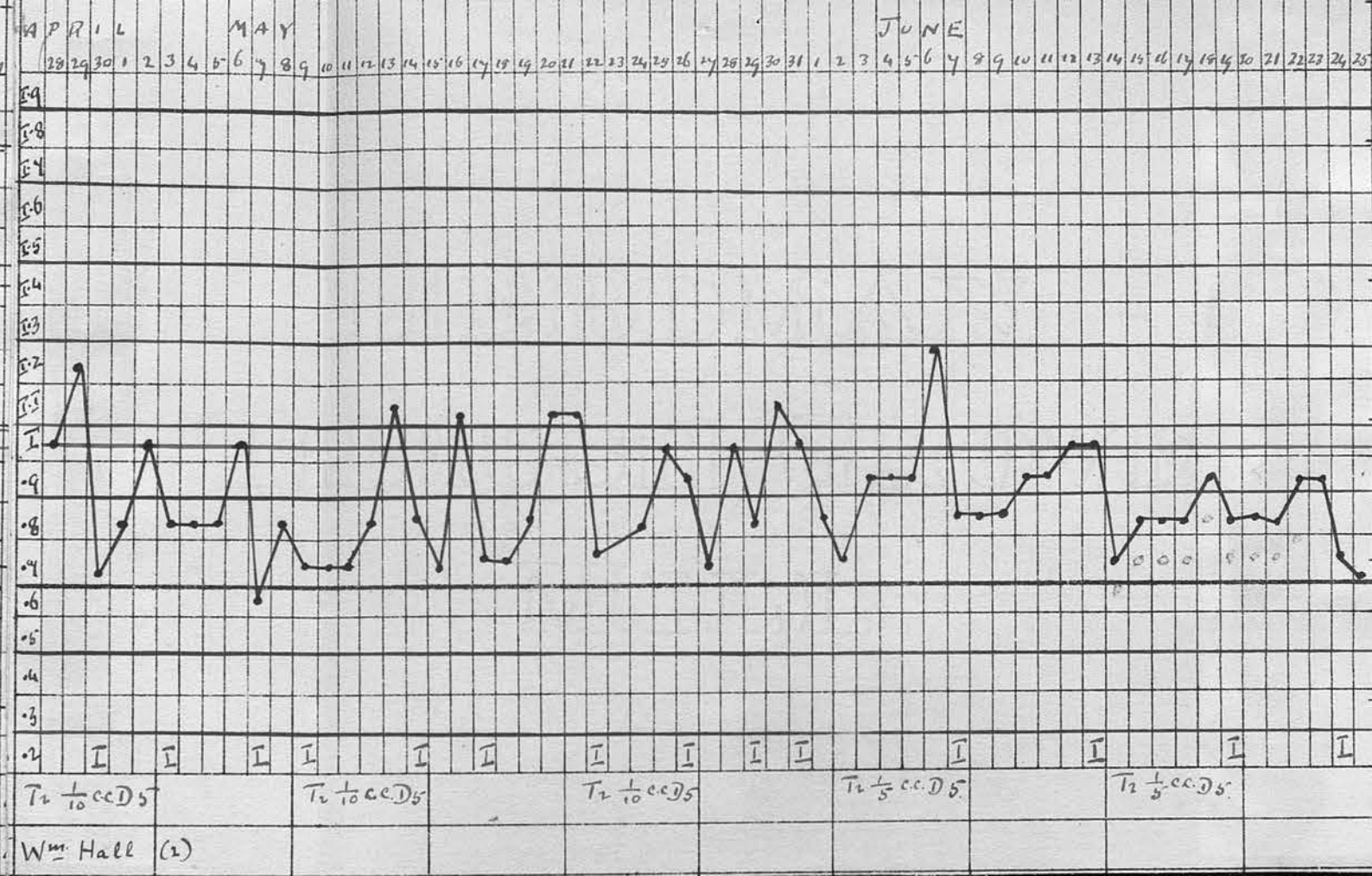
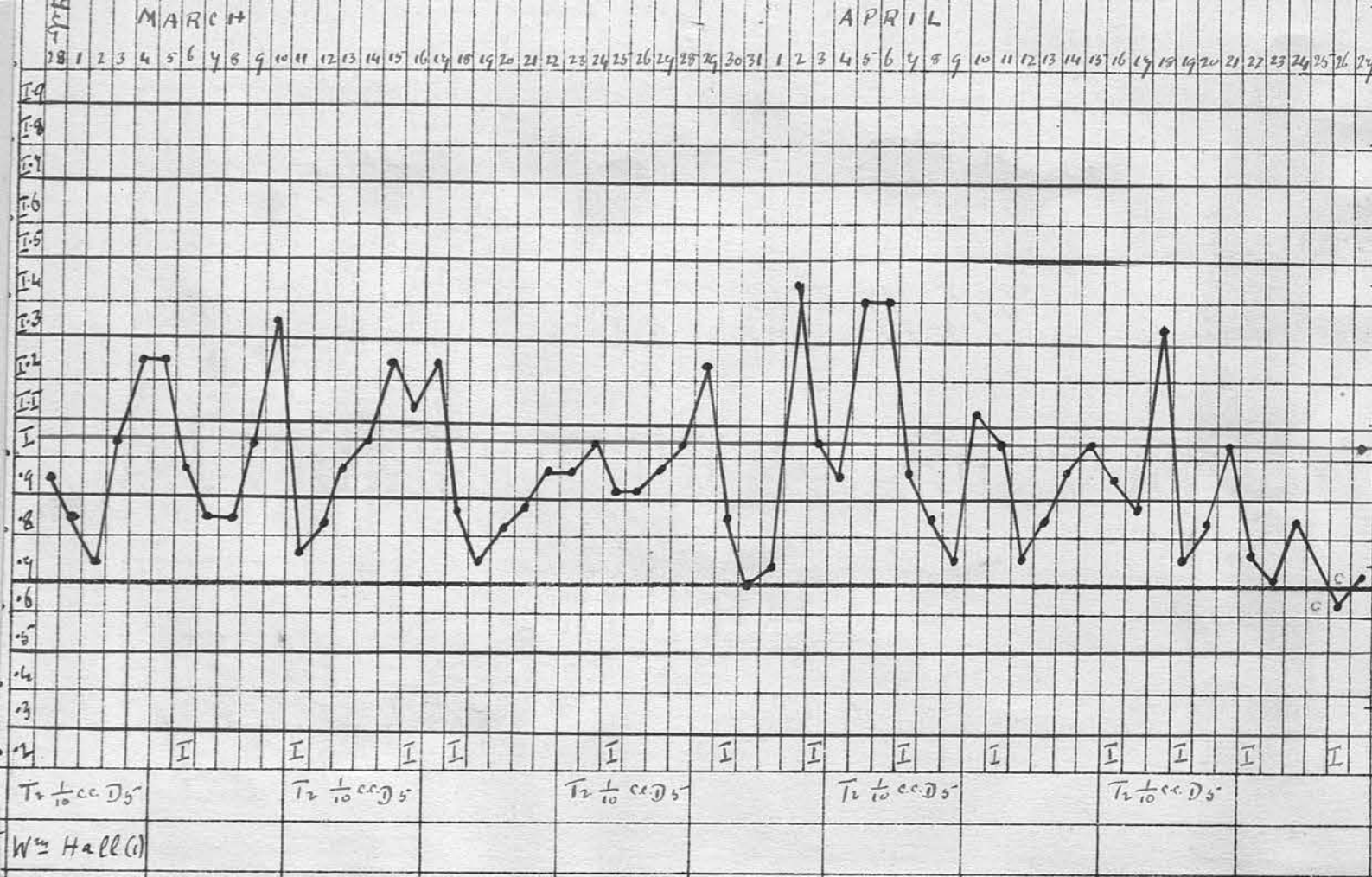
The following shew the effect of small doses injected during a fall in the opsonic reading.

Chart 7.

M. K. age 24, Case of tubercular glands in neck without pulmonary complication. Strong, healthy subject, with several enlarged glands below mastoid process on the left side, and a chain of smaller glands along the anterior border of the left sterno-mastoid muscle. The "glands" had been there, since childhood. The lower series had caused no recent trouble, but the upper were liable to occasional attacks of inflammation and were disfiguring. The case was a very suitable one for tuberculin, there being no complications. Patient had already undergone a course of open-air treatment of about one year's duration, without any effect on the disease. The index was investigated daily for a fortnight before injections were commenced and found to be subnormal from .65 to 1. Injections were commenced on the 8th March. On referring to the chart, it will be seen, an /

an immediate rise to 1.5 followed, the first injection of $\frac{1}{10}$ c.c. D5. The principle of injecting at the commencement of a fall was followed. A second injection was required three days later, which ultimately increased the opsonic value to 1.55. A third dose five days later realised 1.75. A rapid fall resulted, and thereafter, a fairly uniform reading was recorded, which compared with the pre-injection period is distinctly higher. The glands, particularly the large ones, below the mastoid process meantime had become swollen and softened, and they were painful. It will be noticed from the end of April to the middle of June inclusive, the opsonic value tends to become subnormal and uninfluenced by the injections. During this period, pus formation appeared probable in one of the upper glands, the largest of this series. The question of a cutting operation was considered. However, it was decided to increase the dose. Accordingly, on 5th June $\frac{1}{5}$ c.c. D5 was injected with no appreciable effect, in raising the index. A second dose of similar strength was given five days later. It was not until a third dose /

dose had been required, that the index re-acted satisfactorily, 1.2 being recorded. $\frac{2}{5}$ c.c. D5 was given on 20th June and thereafter continued when necessary. The effect of this increase in dose has been to raise and maintain the opsonic value well above control. At the same time and co-incident with the latter, a pronounced improvement set in in the glandular condition. Resolution was rapid. The smaller glands disappeared or became non-palpable. The swelling and congestion of the larger decreased to a marked extent. These could now only be detected by palpation. Unfortunately, at this point, treatment was interrupted, patient leaving hospital. I saw this patient four months later. The condition was then stationary and the appearances were as before, at the point when treatment was stopped. She has recently re-commenced injections, $\frac{1}{2}$ c.c. D5 once weekly. The constitutional effects of small injections in this case were a slight rise in temperature from 97° on day of injection to $98 - 99^{\circ}$ on the day following, and headaches. Patient had always suffered from these. On day following injection, they /



they were aggravated.

Chart 8.

W.F.H. age 28, admitted with slight pulmonary disease. There was grave laryngeal mischief; The arytenoid cushions and ary-epiglottidean folds being ulcerated; also loss of substance posteriorly, in both of the true cords. Voice was very hoarse. Nourishment was good. Under open-air treatment, ordinary and raw-meat feeding, patient made a perfect recovery, so far as the pulmonary condition was concerned. The throat lesion, however, shewed little change for the better. Various local measures had been tried, as application of lactic acid to the ulcerated surfaces. Chief reliance was placed in tuberculin. Injections were given during a fall in the index, $\frac{1}{10}$ c.c. D5 being the initial dose. The chart presents during the first two months a tendency to oscillation, which at certain points is considerable. But the reading is seldom low, '6 being recorded on only one occasion. The effect of the vaccine upon the laryngeal parts during this period was pronounced.

General /

General swelling and congestion were marked, the ventricular bands being notably swollen and obscuring the true cords. Temperature was subnormal. Resolution was noticeable about the beginning of May. The disease in the larynx began to undergo a reparative process, cicatricial changes taking the place of the former ulceration and congestion. The index, had meanwhile, settled in the neighbourhood of control. The swing, which was a feature of the previous readings had now steadied. On 7th June, the dose was doubled $\frac{1}{5}$ c.c. D5, being injected. This quantity was continued as required. The latter third of the record shews the result of the increase, a higher and steadier value obtaining. A further increase to $\frac{2}{5}$ c.c. D5 commenced on August 5th. While this case cannot be pronounced a "cure" in so far as it is possible to check and repair such an extensive laryngeal lesion, the method of injecting under control of the index was quite successful. Doubtless, a similar result would have been obtained by injection of small doses at frequent intervals, without opsonic investigation. The charts of cases

7 and 8 are more satisfactory, than those of 3 and 4. It must be borne in mind, however, that the subjects of 7 and 8 were of a more robust constitution and formed excellent material for injection. Furthermore, the one presented no pulmonary complication, the other only comparatively slight. The lesions were local and more amenable to treatment with tuberculin. A recent examination of the larynx of this patient shewed that the previous improvement had been maintained, The disease was checked and cicatrization complete.

REFERENCES.

- Wright - On a method of measuring the bactericidal power of the blood for experimental purposes. (First communication) "Lancet", December 1, 1900.
- Wright - On the quantitative estimation of the bactericidal power of the blood (Second communication.) "Lancet", March 2, 1901.
- Leishman - Note on a Method of Quantitatively estimating the phagocytic power of the leucocytes of the blood. "British Medical Journal", January 11, 1902.
- Wright - A note on the serum reaction of tubercle with special reference to the intimate nature of agglutination reactions generally and to the therapeutic inoculation of the new tuberculin. "Lancet", May 9, 1903.
- Wright - A lecture on the therapeutic inoculation of bacterial vaccines and their practical exploitation in the treatment of disease. "British Medical Journal", May 9, 1903.
- Wright - On some further improvements in the procedure for testing and judging by the naked eye of the agglutinating and bacteriolytic effects exerted by the sera of patients suffering from, or preventively inoculated against typhoid fever,, Malta fever and tuberculous infections. "Lancet", July 25, 1903.
- Wright and Douglas - His experimental investigation of the role of the blood-fluids in connection with phagocytosis. Proceedings Royal Society, Vol. 72, 1903.
- Wright /

- Wright - On certain new methods of blood examination with some indications of their clinical importance. "Lancet", January 21, 1904.
- Wright - Further observations on the role of the and blood fluids in connection with phagocytosis. "Proceedings of the Royal Society," Vol. 73, 1904.
- Wright - On the action exerted on the tubercle and bacillus by human blood fluids, and on the Douglas - elaboration of protective elements in the human organism in response to inoculation of a tubercle vaccine. "Proceedings of the Royal Society", Vol. 74, 1904.
- Wright - A lecture on the Inoculation treatment of Tuberculosis. "Clinical Journal", November 9, 1904.
- Bulloch - On the principles underlying the treatment of bacterial diseases by the inoculation of the corresponding vaccine. "Practitioner" November, 1905.
- Wright - On the possibility of determining the presence and absence of tubercular infection
Reid. - by the examination of the blood and tissue fluids. "Proceedings of the Royal Society" 1905.
- Wright - On spontaneous phagocytosis and on the and phagocytosis which is obtained with the
Reid. - heated serum of patients, who have responded to tubercular infection, or as the case may be, to the inoculation of a tubercle vaccine. "Proceedings of the Royal Society" 1905.
- Lawson - Use of Opsonic Index of blood in diagnosis and treatment of pulmonary tuberculosis.
- Stewart - "Lancet", November 11, 1905.
- Wright /

- Wright - On the general principles of the therapeutic inoculation of bacterial vaccines as applied to the treatment of tuberculous infection. "Lancet", December 2, 1905.
- Lawson - A study of some points in relation to the and administration of tuberculin (T.R.)"
- Stewart - "Lancet", December 9, 1905.
- Watson Cheyne - Professor A. E. Wright's method of treating tuberculosis. "Lancet", January 13, 1906.
- Gray.- - Vaccine Treatment in Surgery. "Lancet", April 21, 1906.
- Bradshaw -A note on the influence of anti-toxic serum on the tuberculo-opsonic index. "Lancet", May 19, 1906.
- Grace - Calvert - Opsonins and opsonic index and their practical value in the treatment of disease, especially in regard to pulmonary tuberculosis in sanatorium patients. "Lancet", November 24, 1906.
- Reyn and Observations on the opsonins, with special Kjer- regard to lupus, vulgaris. "Lancet", Petersen - March 28, 1908.
- Allen - The Opsonic method of treatment. 1907.
- Hewlett - Serum Therapy. 1903.